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Communications

Effectiveness of a commonly-used technique for experimentally reducing plumage UV reflectance

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Ultraviolet (UV) plumage is thought to be sexually selected through intra-sexual competition, female choice and differential allocation. Experimental manipulations of plumage UV reflectance are essential to demonstrate that mate choice or intra-sexual competition are causally related to UV coloration. The most widely-used technique for manipulating UV reflectance in wild birds is the application of a mixture of UV-absorbing chemicals and preen gland fat. However, although this UV reduction technique is commonly used, little is known about the persistence of the treatment and the temporal variation in UV reflectance that it causes. We manipulated the UV crown plumage of wild and captive blue tits *Parus caeruleus*, and took repeated photospectrometric measurements of both UV-reduced and control-treated individuals. Our results show that the UV reduction lasts for at least five days and that the treatment has no negative effects on the survival of wild birds.

Recently there has been a surge of interest in avian colour vision and coloration, especially regarding the significance of plumage ultraviolet (UV) reflectance, which is visible to most bird species but not to their human observers (Hill and McGraw 2006). Descriptive studies have implicated UV plumage and other coloration in sexual selection, through intra-sexual competition (Senar et al. 1993, Siefferman and Hill 2005), female choice (Andersson et al. 1998, Hill et al. 1999) and differential allocation (Linville et al. 1998), but experimental manipulation of coloration is needed to unequivocally demonstrate a causal link between the behaviour of conspecifics and an individual's coloration (Hill 1991, Bennett et al. 1996, Johnsen et al. 1998, Limbourg et al. 2004).

The most widely used technique for manipulating plumage UV reflectance in wild birds involves applying a mixture of UV-absorbing chemicals and duck preen gland fat to the feathers. This technique was first used by Andersson and Amundsen (1997) in bluethroats *Luscinia svecica* and has since been used on several species in both the field and captivity (Table 1). Although these experiments show that conspecifics respond to the treatment, and some studies have given approximate indications of how long the treatment lasts (Johnsen et al. 1998, Limbourg et al. 2004), there has, remarkably, been no detailed study of the time course of the UV reduction effect. Thus we have little idea of how the coloration varies through time after the treatment. This information is particularly pertinent in studies which aim to measure a response to the UV manipulation several days after the application of the treatment. For example, crown UV reflectance in male blue tits *Parus caeruleus* has been manipulated before the start of laying by the female after which the sex ratio of the subsequently-laid clutch was measured (Sheldon et al. 1999, Korsten et al. 2006). In blue tits, successive eggs of a clutch are laid daily over a period of about 10 d (mean clutch size: 10.9 ± 1.7 SD; Korsten et al. 2006). Thus, depending on the temporal variation in UV reflectance, the sex of individual eggs in a clutch may have been determined when the male differed considerably in appearance. In another study on blue tits, UV reflectance of males was reduced 2 d before hatching and again when the chicks were 7 d old. Subsequently, female provisioning behaviour was

Table 1. Studies manipulating plumage UV reflectance using mixtures of UV-absorbing chemicals and preen gland fat.

Species	Captive/wild	Time between treatment and measurement of response (in d)	Response to treatment	Description of response	Reference
Blue tit *Parus caeruleus*	Wild	10 ± 5.2 SD	Yes	Females adjust offspring sex ratio in response to male UV reduction	1
Blue tit	Wild	3/7	Yes	Females feed their young less when paired to UV-reduced males	2
Blue tit	Wild	8.0 ± 6.5 SD/4.4 ± 2.9 SD	Yes/No	Females adjust offspring sex ratio in response to male UV reduction in 1 of 2 years	3
Bluethroat *Luscinia svecica*	Captive	<1	Yes	Females discriminate against UV-reduced males in choice test	4
Bluethroat	Wild	Variable: ca 7–20	Yes	UV-reduced males have lower (extra-pair) mating success	5
Pied flycatcher *Ficedula hypoleuca*	Captive	<1	Yes	Females discriminate against UV-reduced males in choice test	6

1. Sheldon et al. 1999; 2. Limbourg et al. 2004; 3. Korsten et al. 2006; 4. Andersson and Amundsen 1997; 5. Johnsen et al. 1998; 6. Siitari et al. 2002.

measured when the chicks were 10 and 14 days old (Limbourg et al. 2004; see Johnsen et al. 2005 for a similar experiment using marker pens instead of UV-absorbing chemicals). Again, male coloration during the observations of female behaviour could have differed considerably from that immediately after treatment. Clearly, knowledge of the temporal changes in the effect of UV-reduction treatment would facilitate the successful application and correct interpretation of these kinds of experiment.

We therefore investigated how UV coloration varied with time after treatment in both wild and captive birds. We studied blue tits, because their crown UV coloration is one of the most extensively investigated UV-reflecting plumage ornaments (Andersson et al. 1998, Hunt et al. 1998, Sheldon et al. 1999, Delhey et al. 2003, Limbourg et al. 2004, Johnsen et al. 2005, Hadfield et al. 2006, Korsten et al. 2006) and the most frequent subject of manipulation using UV-absorbing chemicals (Table 1).

Methods

General

We caught wild male blue tits in the period from nest building to hatching at De Vosbergen, The Netherlands (see Korsten et al. 2006 for details) during 2002 and 2003, and manipulated their crown UV reflectance (42 UV-reduced males, 43 controls). Crown UV reflectance was measured immediately before and after treatment (=day 0). 70 males were recaptured and remeasured during chick provisioning, most of them (65 males) either 7–14 d (8 UV-reduced males, 8 controls) or ≥28 d after the initial treatment (26 UV-reduced males, 23 controls).

In addition, 4 male blue tits were captured at Westerheide (The Netherlands) in November 2002. They were held together in a large outdoor aviary (ca 2 × 4 × 3 m) at the Netherlands Institute of Ecology (NIOO) in Heteren and fed *ad libitum* with standard bird food. Their crown UV reflectance was manipulated (all reduced) and measured immediately before and after treatment (=day 0), and on days 1–7, 9, 12 and 16. Males were subsequently released at the capture site.

Crown UV treatment and measurements

We reduced UV reflectance of the crown feathers using a 40/60% (by weight) mixture of duck preen gland fat (which is commercially available and used as fishing fly dressing; purchased at Euro-Fly, Paris, France), and UV-absorbing chemicals (Parsol 1789 and Parsol MCX (50% of each by weight; Roche, Basel, Switzerland; Andersson and Amundsen 1997, Johnsen et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004, Korsten et al. 2006). As a control, we applied pure duck preen gland fat (Johnsen et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004, Korsten et al. 2006).

We measured the reflectance of the crown feathers using a USB-2000 spectrophotometer and DH-2000 deuterium-halogen light source (both Avantes, Eerbeek, The Netherlands). For more details of measurement and processing of the reflectance spectra see Limbourg et al. (2004) and Korsten et al. (2006). We calculated 'UV chroma' as the sum of reflectance between 320–400 nm divided by the sum of reflectance between 320–700 nm ($R_{320-400}/R_{320-700}$) following previous studies (Sheldon et al. 1999, Delhey et al. 2003, Limbourg et al. 2004, Korsten et al. 2006). UV chroma is an important predictor of male attractiveness in blue tits (Andersson et al. 1998, Sheldon et al. 1999, Limbourg et al. 2004).

We also measured crown reflectance of unmanipulated males (n = 111) and females (n = 169) at De Vosbergen during the 2001–2003 breeding seasons.

Results

Effect of UV manipulation on crown coloration of wild males

Both UV-reduced and control-treated feathers became slightly more glossy after the treatment, but otherwise looked the same to the human observer. The gloss caused a small uniform increase in reflectance for both treatments (Fig. 1). The UV reduction treatment clearly reduced the reflectance between 320–400 nm, whereas the control treatment did not (Fig. 1). So treatment reduced UV chroma by 38% compared to pre-treatment values (paired t-test: $t = 28.90$, $df = 41$, $P < 0.001$; Fig. 2), a value 24% and 6% below the natural range of UV chroma for males and females, respectively (Fig. 2). UV chroma was not affected by the control treatment ($t = 1.63$, $df = 42$, $P = 0.11$; Fig. 2). The change in spectral profile (Fig. 1) resulting from the UV-reduction treatment also increased the wavelength at peak reflectance (mean $\lambda_{max} \pm SE$, before: 381 ± 2.2 nm, after: 418 ± 1.1 nm; paired t-test:

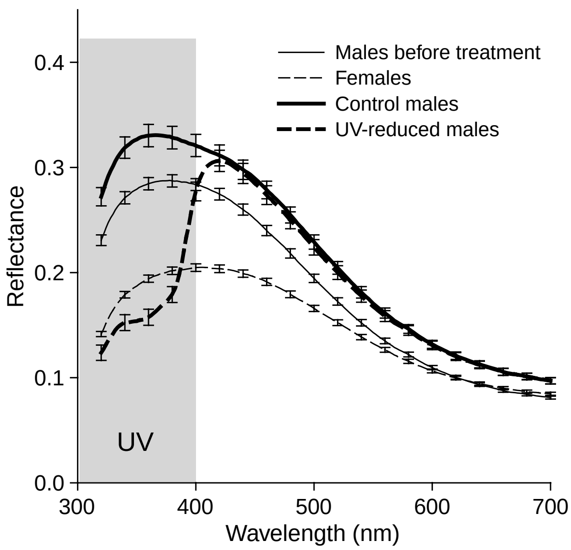

Fig. 1. Mean reflectance curves of the crown plumage of wild male blue tits before manipulation (n = 85), after UV reduction (n = 42), and after control treatment (n = 43). The mean reflectance curve for unmanipulated females (n = 169) is shown for reference. Standard errors are depicted at 20-nm intervals. The shaded area indicates the UV part of the spectrum.

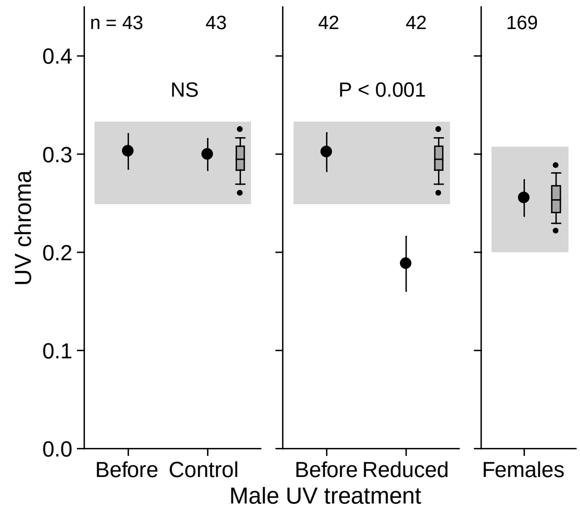

Fig. 2. Mean UV chroma of crown plumage of wild male blue tits before and after UV reduction and control treatment. The mean UV chroma of unmanipulated females (n = 169) is shown for reference. Whiskers indicate standard deviations. Shaded areas indicate natural ranges of UV chroma of males (n = 111; range 0.249–0.333) and females (n = 169; range: 0.200–0.307). Box plots on the right of each panel show the variability of natural UV chroma in males and females (depicted are the median and the 5th, 10th, 25th, 75th, 90th and 95th percentiles).

$t = -19.70$, $df = 41$, $P < 0.001$). The control treatment caused a smaller but significant decrease in λ_{max} ($\lambda_{max} \pm SE$, before: 377 ± 1.8 nm, after: 368 ± 2.0 nm, paired t-test: $t = 4.85$, $df = 42$, $P < 0.001$). Pre- and post-treatment UV chroma of individual males were strongly correlated in both treatment groups (UV-reduced: $r = 0.47$, $n = 42$, $P = 0.002$; control: $r = 0.72$, $n = 43$, $P < 0.001$; Fig. 3), and the slopes of the relationships did not differ between the groups (ANCOVA with UV chroma after treatment as response variable: UV treatment × UV chroma before treatment: $F_{1,81} = 0.007$, $P = 0.93$; Fig. 3).

Temporal change of UV reduction in captive and wild males

The effect of the UV-reduction treatment in captive birds diminished over time, being most rapid directly after application (Fig. 4a). Although the treatment initially decreased UV chroma to unnaturally low values, average UV chroma of UV-reduced males was within the natural range again two days after treatment (Fig. 4a), and the reduction in UV chroma (compared to pre-treatment values) was no longer significant 6 days after treatment (Fig. 4a). Wild birds showed a similar pattern (Fig. 4b), although UV-reduced males still had significantly lower UV chroma than control

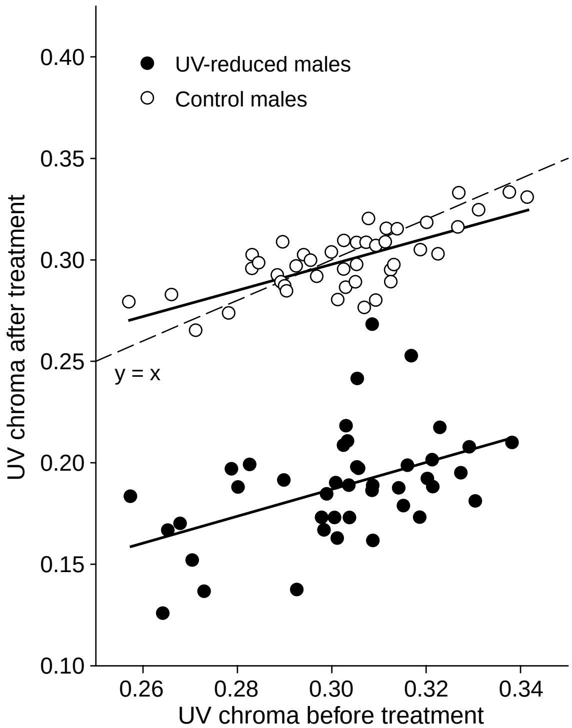

Fig. 3. UV chroma of crown plumage of individual wild male blue tits before and after UV reduction (n = 42) or control treatment (n = 43). Solid lines are linear regressions for each treatment group separately.

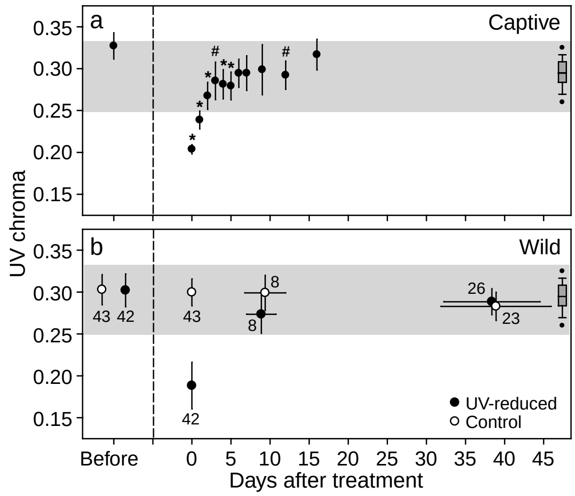

Fig. 4. Temporal changes of crown UV chroma after UV-reduction treatment of (a) captive and (b) wild male blue tits. a. Mean UV chroma ± SD of 4 individual UV-reduced males who were repeatedly measured. Differences in UV chroma from pre-treatment values were tested with one-tailed paired t-tests (#P < 0.05; *P < 0.01). b. Mean UV chroma values ± SD of UV-reduced and control-treated males are shown before and immediately after treatment, and for manipulated males recaptured 7–14 days or > 28 days after treatment. Numbers indicate sample sizes. Shaded areas in both panels (a, b) indicate natural ranges of male UV chroma (n = 111). Box plots on the right of each panel show the variability of natural UV chroma in males (depicted are the median and the 5th, 10th, 25th, 75th, 90th and 95th percentiles).

males 7–14 days after treatment ($t = -2.36$, $df = 14$, $P = 0.034$; Fig. 4b), while both values were within the natural range (Fig. 4b). The difference between the UV chroma of UV-reduced and control males had disappeared in individuals recaptured ≥ 28 days after treatment ($t = 1.19$, $df = 47$, $P = 0.24$; Fig. 4b). There was no difference in survival to the following breeding season between treated and untreated males (treated males: 37.6%, n = 85; untreated males: 39.2%, n = 74; Fisher's exact test: $P = 1.0$), or between UV-reduced and control males (UV-reduced: 35.7%, n = 42; control: 39.5%, n = 43; Fisher's exact test: $P = 0.84$).

Discussion

Our results confirm that the application of a mixture of preen gland fat and UV-absorbing chemicals reduces UV reflectance, whilst pure preen gland fat can serve as an adequate control. The UV reduction effect diminishes rapidly shortly after the treatment, but is still detectable after 5 days in captive birds, and 7–14 d in wild birds. Importantly, mean UV chroma values are outside the natural range for only a short period (less than 2 d in captive males), partly refuting previously raised concerns that manipulated birds were outside the natural range (Johnsen et al. 2005, Hadfield et al. 2006). We do not know how conspecifics perceive the effect of the UV reduction treatment. For example, they might respond to discordance between the coloration of different areas of plumage (Sheldon et al. 1999) or to temporal variation in UV reflectance (Limbourg et al. 2004). Nevertheless, we wish to emphasize that these experiments demonstrate that information contained in the UV part of the spectrum is causally involved in intra-specific communication.

The treatment had no undesired side-effects in the wild (and captive) blue tits. In spite of their changed appearance, most manipulated wild males re-established contact with their mates immediately after release (P. Korsten pers. obs.) and the treatment never led to divorce (see Korsten et al. 2006). The treatment was reversible (no UV reduction effect detectable after approximately 28 d) and had no negative effects on the chances of survival to the following breeding season.

Marker pens have also recently been used to successfully manipulate UV plumage coloration (Ballentine and Hill 2003, Johnsen et al. 2005), and can produce an increase, as well as a decrease, in UV reduction, although duration of the treatment effects has not yet been investigated.

In conclusion, mixtures of UV absorbing chemicals and (preen gland) fat offer an excellent tool for manipulating the UV reflectance of plumage. Our results will add considerably to the usefulness of studies using this technique, by underlining the need for careful planning, possibly including re-application of the treatment (Limbourg et al. 2004), because of the short-term nature of the UV reduction, and by revealing the time course of variation in UV coloration in relation to the behavioural responses that are measured.

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